A GLYCOSPHINGOLIPID SHARING REACTIVITY WITH BOTH WHEAT GERM LECTIN AND 'CARCINOEMBRYONIC ANTISERA (GOLD)': PARTIAL IDENTITY OF THESE REACTIVE SITES*

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1. Introduction

Wheat germ lectin (WGL) preferentially agglutinates transformed cells [1–4], although the underlying mechanism is not exactly known. This agglutination can be inhibited by a glycolipid fraction derived from various tissues and cells, including human adenocarcinoma, Krebs ascites hepatoma, and transformed BHK cells [4,5]. A glycoprotein with similar receptor activity was also isolated from Novikoff tumor cell surfaces [6]. The degree of inhibition obtained by glycolipid of transformed cells was higher than that of comparable normal cells, and a Smith-degraded glycolipid fraction of normal cells or tissue exhibited a much greater degree of reactivity than before degradation [4]. These findings are in accordance with the observation that Smith-degraded** Lewis glycolipids having GlcNAc at the non-reducing terminal showed an extremely high reactivity to WGL [7]. Carcinoembryonic antigen (CEA) of human digestive system, first described by Gold and Freedman [8] as a specific antigen for tumors of digestive organs and embryonic gastrointestinal tissue, has been identified as glycoprotein [9,10].

In this communication we wish to report a purified glycosphingolipid showing strong reactivity to WGL as well as to anti-CEA antiserum. The glycolipid was isolated from human A erythrocytes and from human adenocarcinoma. A number of immunodiffusion analyses indicated that the reactive sites for 'anti-CEA' antiserum and those for 'WGL' are at least partially identical.

2. Materials and methods

Glycolipid fractions of tumor tissue [7] or of erythrocytes [13,14] were further fractionated by Folch's partition, followed by DEAE-cellulose chromatography [15], according to the method for obtaining 'blood group glycolipids' described previously [14]. Each fraction was examined by gel diffusion analysis with WGL or with anti-CEA as follows: Glycolipid (100 μg) was dissolved in 100 μl of physiological saline on heating at 60–80°C and was placed in a 'cleaner bath' with sonicator for a few minutes. Twenty to thirty microlitres was put in a well of plastic template placed on 0.5% agarose in physiological saline and reacted with 10–20 μl of a 1% solution (in physiological saline) of WGL or anti-CEA, according to the method of Sharples and LoGrippo [16].

WGL was prepared according to Burger and Goldberg [2]; part of the preparation used in this experiment was purified by affinity chromatography [17] and was kindly donated by Dr. Nathan Sharon,

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** *Abbreviations:* CEA, carcinoembryonic antigen of Dr. Phil Gold; this term is used according to his definition [6]; anti-CEA, antiserum directed against carcinoembryonic antigen of Gold; the antiserum showed a major monospecific precipitin line with CEA; Smith-degraded, product of Smith degradation [11], if applied to micelles of glycolipids with low concentration of periodate, often resulting in degradation of non-reducing terminals of carbohydrate chain [12].

Weizmann Institue of Science, Israel. Both preparations showed an identical precipitin reaction with a specific glycolipid and glycoprotein. The precipitin reaction was enhanced in the presence of a trace amount of Ca^{2+} and Mn^{2+} (10^{-5} M)

Anti-CEA was kindly donated by Dr. Phil Gold, Montreal General Hospital, and by Dr. Jack Haverback, University of Southern California. The fraction with positive reaction was further purified by this layer chromatography (TLC) on silica gel H and then acetylated in pyridine and acetic anhydride. The acetylated compounds were further separated on TLC as previously described according to the method applied for extensive purification of H-glycolipid and other fucose glycolipids [18]. The purified glycolipids as acetate were deacetylated in chloroform–methanol–sodium methoxide [18] and analyzed. The carbohydrate composition was analyzed according to the method described previously [6].

Glycoprotein with positive CEA reaction was prepared from human adenocarcinoma by the method of Gold et al. [8]. Purified CEA was a gift from Dr. Phil Gold and from Dr. Jack Haverback. *NN'*-diacetylchitobiose was a gift from Dr. T. Osawa, Tokyo University, Faculty of Pharmaceutical Science.

3. Results

The purified glycosphingolipid obtained by acetylation procedure gave a strong precipitin reaction with both WGL and with anti-CEA. This glycolipid inhibited hemagglutination caused by WGL; 6–12 μg of glycolipid can inhibit hemagglutination caused by three hemagglutinating doses of WGL. Trials to separate components that reacted with WGL from components that reacted with anti-CEA were all unsuccessful. Two samples of glycolipids prepared from human erythrocytes, two samples prepared from human adenocarcinoma (liver metastatic deposit from laryngeal and cecal cancer), and four samples of CEA glycoprotein were all equally reactive to both WGL and anti-CEA. The two reactivities were demonstrated to be 'identical' or 'partially identical' according to the precipitin pattern on double diffusion agarose plates.* An example is shown in fig. 1A and 1B. The precipitin line with WGL was completely suppressed by inclusion of 1 mM of *NN'*-diacetylchitobiose in agarose plates, in agreement with previous reports [2], whereas that with anti-CEA was only slightly suppressed even at 50 mM concentration of *NN'*-diacetylchitobiose in agarose (experiment with anti-CEA provided by Dr. J. Haverback). This agreed with the previous observation that blood group hapten

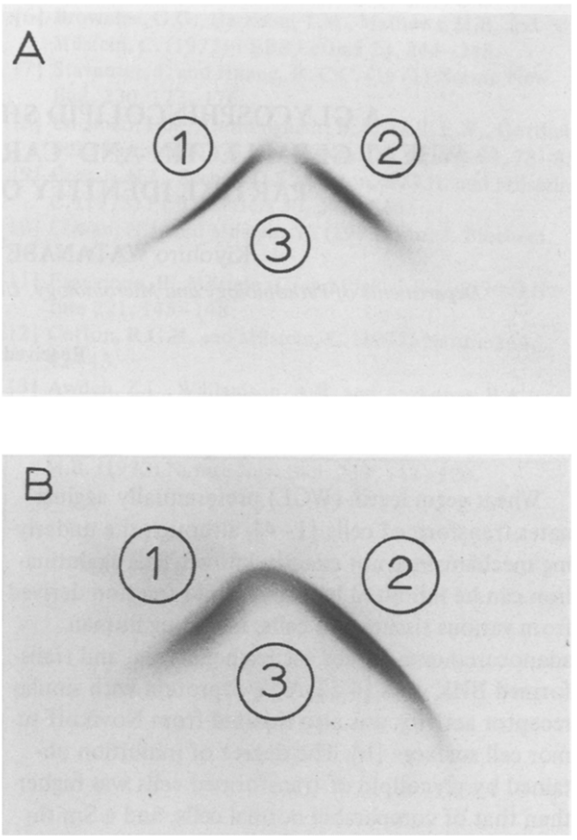

Fig. 1. Precipitin reaction in agar gel showing a shared reactivity between wheat germ lectin and anti-carcinoembryonic antigen: A, 1 WGL; 2 anti-CEA; 3 purified glycolipid hapten. B, 1 WGL; 2 anti-CEA; 3 purified CEA glycoprotein.

* A preliminary study with *Dolichos biflorus* and anti-A serum indicated that the Ouchterlony criterion [24] can be valid in interpreting the precipitin line formed by lectin and by immunoglobulin, although no extensive data are available. The Ouchterlony criterion is also difficult to apply when a crude glycolipid antigen is used, as the pattern is greatly influenced by micellar aggregation [25]. The pattern shown in fig. 1 is produced by the purified glycolipid antigen.

inhibition was much more effective in reaction with lectin than with immunoglobulin, i.e. blood group A and H reactivities with various lectins were readily inhibited by immunodominant monosaccharide or oligosaccharide. The same reactivities with specific immunoglobulins were more difficult to inhibit by monosaccharide or oligosaccharide [19]. The participation of GlcNAc in determining CEA specificity was already suggested by Gold and his colleagues [21].

The purified glycolipid isolated from blood group A erythrocytes had fucose, glucose, galactose, glucosamine and galactosamine in the molar ratio of 1.0: 0.9:2.1:0.9:1.7. Smith degradation caused destruction of fucose and galactosamine, but the reactivities to WGL and anti-CEA were intensified after degradation, suggesting that both activities reside on the internal structure of the carbohydrate chain, which is composed of *N*-acetylglucosamine (possibly 2 moles) and galactose.

Purified glycolipid was obtained with an extremely poor yield (a few mg from 500 g of membrane preparation); further structural study is inconvenient at the present time. The quantity of this glycolipid present in blood group A erythrocyte membrane was greater than that in blood group B or O erythrocytes, although quantitative data is not available at the present time.

4. Comments

The reactivities of glycolipid to WGL and to anti-CEA were not demonstrated when crude glycolipid fractions were tested; it was only possible after the glycolipid was freed from a large excess of contamination. This situation is the same as that for H-activity of H-glycolipid, which was difficult to demonstrate with anti-H reagents (eel serum or *Ulex europeus* extract), unless it was purified [18]. The results of double diffusion assay indicated a strong possibility that the reactive sites to anti-CEA also react to WGL. However, not all the structures that react to WGL are reactive to anti-CEA. A ceramide trisaccharide (*N*-acetylglucosaminyl β1→3-galactosylβ1→4-glucosylceramide) isolated from the degradation product of blood group glycolipid was strongly reactive to WGL but was devoid of any reactivity to anti-CEA. WGL must have a wide specificity that is directed to a number of structures made of β-*N*-acetylglucosamine, whether they are located internal or external to the carbohydrate chain. Of a number of WGL reactive sites, only a few limited structures must be reactive to anti-CEA. The glycolipid isolated showed very similar properties on TLC as blood group glycolipids in R_f values on TLC and chemical composition. It is extremely interesting to compare this data to that of Simmons and Pearlman, who recently described that CEA glycoprotein is essentially an 'incomplete blood group glycoprotein' [20].

The presence of a greater quantity of the glycolipid hapten in A erythrocytes than in B or O erythrocytes coincides with the observation that WGL reacts more strongly with blood group A substance than with B or O substances [5] and also with the observation that anti-A antibody combines with CEA giving some cross-reactivity between CEA and blood group substance A [21]. These findings strongly suggest that synthesis of the structures which determine the reactivity to WGL or to anti-CEA can be controlled by regulatory genes that are functionally related to regulation of A-gene expression. They could be located at close proximity to each other on the chromosome.

It is interesting to note that expression of blood group A or B antigens closely relate to the developmental stages; they appear at the very early stage in primitive epithelial organs, disappear during developmental stages, and reappear after the developing organs reach their final adult form [23]; they disappear when transformed into tumor cells [5]. Their expression is opposite to the behavior of CEA.

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